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Open-label, non-randomised, inter-individual dose escalation of ZK 304709 with the evaluation of safety, tolerability, pharmacokinetics, oral bioavailability and orientating efficacy after daily administration in patients with advanced cancer (7 d treatment and 14 d recovery)

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ABSTRACT

Purpose: The primary objectives of this study were to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of ZK 304709, a novel multi-targeted growth inhibitor (MTGI™), in man. Secondary end-points included safety evaluation, tolerability, pharmacokinetic profiling and assessment of response using standard and novel surrogate pharmacodynamic end-points.

Materials and methods: Patients ($n = 40$) with advanced solid malignancies were treated with ZK 304709, administered orally once daily for 7 d with 14 d recovery. Doses were escalated in sequential cohorts of three patients with expansion to 6–7 patients should a dose-limiting toxicity occur.

Results: ZK 304709 was safely administered up to 360 mg. However, above 90 mg blood concentrations increased only slightly. As this dose was not deemed likely to result in meaningful pharmacologic or clinical activity, the trial was stopped before the MTD was ascertained. It was therefore not possible to make a reliable assessment of efficacy or pharmacodynamic end-points.

Conclusions: Due to the lack of further increment in blood concentrations above a dose of 90 mg, which was felt from previous animal studies to be unlikely to result in meaningful pharmacologic or clinical activity, this study was stopped early.

1. Introduction

ZK 304709 is a first-in class oral Multi-Target Tumour Growth Inhibitor (MTGI™) that blocks tumour cell proliferation and induces apoptosis *via* inhibition of cell-cycle progression and tumour-induced angiogenesis. It inhibits cyclin-dependent kinases (CDKs) 1, 2, 4, 7 and 9; vascular endothelial

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growth factor receptor-1, -2, and -3 tyrosine kinases (VEGF-R1TK, -R2TK, -R3TK) and platelet-derived growth factor receptor beta tyrosine kinases (PDGF-R beta TKs). This profile suggests that ZK 304709 inhibits tumour growth and systemic spread *via* cell cycle arrest and apoptosis induction as well as *via* inhibition of tumour angiogenesis.[1]

Pre-clinical *in vitro* and *in vivo* results indicated that ZK 304709 was an innovative and potentially more effective alternative to current treatments for solid tumours. *In vitro* anti-tumour potential was demonstrated in a panel of 25 human tumour cell lines. *In vivo* anti-tumour activity was demonstrated in a broad panel of human tumour xenograft models in nude mice. In these models ZK 304709, in particular on xenografts derived from estrogen-independent breast cancer, androgen-independent prostate cancer, pancreatic cancer, renal cell cancer, colorectal cancer and acute myelogenous leukaemia (AML), activity was frequently superior to that of standard cytotoxic chemotherapies (e.g. doxorubicin and paclitaxel) and to that of CDK inhibitors currently under clinical development (e.g. flavopiridol and R-roscovitine).[1] After intra-gastric administration, retardation of tumour growth was observed at daily dose levels of 30 mg/kg bw. (approximately 90 mg/m^2 BSA) in some models. At dose levels $\geqslant$75 mg/kg body weight. (approximately 225 mg/m^2 BSA) complete growth inhibition or tumour regression could be shown. It did not permeate the blood–brain barrier, and was completely eliminated *via* kidney and liver within one week having undergone little metabolism. Expected toxicities from pre-clinical studies included reversible gastrointestinal, renal and haematologic toxicities.

We conducted this first-in-man phase I study to determine the MTD of ZK 304709 when administered daily for 7 d with 14 d recovery. In addition to the standard secondary end-point of tumour response, we hoped to explore novel surrogate pharmacodynamic end-points, by measuring biomarkers already known or postulated to be involved in the pathogenesis of cancer and/or related to the mechanism of action of ZK 304709.

2. Materials and methods

The primary objectives of this study were to determine the maximum tolerated dose (MTD) and the dose limiting toxicities (DLTs) of ZK 304709 in patients with advanced solid tumours. The secondary objectives included evaluating safety and tolerability, establishing the pharmacokinetic profile including absolute oral bioavailability of ZK 304709 and measuring tumour response and evaluating biomarkers in tumour and blood samples.

2.1. Study design

This was a multi-centre, open-label, non-randomised, inter-individual dose escalation of ZK 304709 with the evaluation of safety, tolerability, pharmacokinetics, absolute oral bioavailability and efficacy after 7 d once daily treatment and 14 d recovery. The starting dose was 15 mg with subsequent escalations depending on prior cohort toxicity. Adverse events were evaluated using the National Cancer Institute Common Toxicity Criteria, version 2.0 guidelines.

2.2. Patient eligibility

Patients aged $\geqslant$18 years with histologically confirmed advanced solid tumours refractory to standard therapy or for whom no effective therapy was available were eligible. Inclusion criteria included written informed consent, baseline WHO performance status score of 0–2, adequate function of major organs, life expectancy of at least 3 months, absolute neutrophil count $>1.5\times10^9$/l, haemoglobin >9 g/dl, platelets $>100\times10^9$/l, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) $<2.5\times$ upper limit of normal (ULN), $<5\times$ ULN for patients with liver metastases, serum bilirubin $<1.5\times$ ULN, serum creatinine $<1.5\times$ ULN and for women of childbearing potential a negative pregnancy test within 24 h prior to first administration of ZK 304709. Exclusion criteria included concurrent severe and/or uncontrolled disease, myocardial infarction within 6 months before the start of study, unstable or uncontrolled hypertension, chronic renal disease, active uncontrolled infection, obstructive lung disease, hyperchloremic acidosis, liver disease (i.e. hepatitis, cirrhosis), human immunodeficiency virus (HIV), hepatitis B or C infection, treatment with investigational drugs, such as chemo-, biological- or immuno-therapy treatment less than 4 weeks prior to screening, or surgery or radiotherapy within 2 weeks of screening or any patients who had not recovered from the toxic effects of such therapy, brain metastasis or primary brain tumours, chronic enteric disease which could compromise study drug absorption, female patients who were pregnant or breastfeeding or patients of reproductive potential not employing effective contraception.

2.3. Treatment plan

ZK 304709 was supplied by Bayer Schering Pharma. After obtaining informed consent and performing screening examinations, eligible patients were included into consecutive dose groups of 7 patients maximum. Initially 3–4 patients were enroled in a dose group. ZK 304709 was administered by mouth for 7 consecutive days followed by a 14-d recovery phase when no study drug was administered. Patients were fasting for 3 h before and 1 h after study drug administration. Patients who had taken fewer than 7 daily treatments or had taken 7 daily treatments within a period which was longer than 10 d in their first treatment course for reasons other than drug-related toxicities were replaced. The recovery phase could be extended to 4 weeks if the following findings were still present: platelets $<100\times10^9$/l, ANC $<1.5\times10^9$/l, non-haematologic toxicities not recovered to grade 1 or less (except alopecia). Patients who developed grade 3 or 4 toxicities prior to the DLT assessment and considered not to be drug-related were also replaced. In the absence of disease progression six cycles of treatment were planned. However, study drug administration could be continued beyond 6 cycles at the investigator's discretion. Following the last treatment, safety and tolerability of patients were to be observed for an additional period of 4 weeks.

The starting dose level of 15 mg/d was selected based on studies in dogs and rats according to the recommendations covered in the draft FDA guidance: 'Estimating the safe starting dose in clinical trials for therapeutics in adult healthy

volunteers'. Considering the assumed MTDs from these toxicological studies (210 mg/m^2 for dogs and 180 mg/m^2 for rats), one-tenth of the lowest MTD is suggested as the clinical starting dose per m^2 for trials in humans. This results in a dose level of 18 mg/m^2, that is 36 mg per administration for a BSA of 2 m^2. As an additional measure of precaution, it was decided to start with a dose half of that estimated on the basis of toxicological data (15 mg per day/patient). Considering *in vitro* and animal data and assuming a human blood/plasma ratio of about 10, efficient tumour inhibition should be achieved in patients at whole blood threshold concentrations higher than 3–5 μg/ml (corresponding to about 0.4 μg/ml [1 μM] in plasma). Based on *in vitro* studies, daily tumour exposure should last for at least 7 h.

Dose levels were to be initially escalated by 100% of the previous dose according to a previously described accelerated titration model[2] to reach potentially efficacious dose levels as soon as possible. Dose escalation for the next dose level was to be limited to approximately 50% when a patient experienced grade 2 or greater toxicity in the first course which was at least possibly related to the study drug. The dose escalation for each subsequent dose group was to be approximately 33% of the preceding dose until DLT occurred, and the MTD could be defined. By these means, both the risk of exposure to excessive toxicity and the number of patients treated at presumably less effective dose levels were expected to be limited. Subsequently, the protocol was amended to allow 100% dose escalation to occur if, at the next higher dose level, the toxicity that had previously limited the dose escalation did not recur, recurred with an intensity of less than grade 2 or was assessed as unlikely to be related to the study drug. Intra-patient dose escalation was not permitted.

2.4. Dose limiting toxicity

Patients were evaluated at regular intervals for adverse events, laboratory (haematology, chemistry, urinalysis) and electrocardiogram (ECG) abnormalities. DLTs were defined as the following study-drug-related events (possibly, probably or definitely related) occurring during course 1: grade 4 neutropaenia for more than 5 d, or febrile neutropaenia (absolute neutrophil count [ANC] $< 1.0 \times 10^9$/l and body temperature >38.5 °C); thrombocytopaenia (platelet count $<25 \times 10^9$/l); grade 3 or 4 non-haematologic toxicity (except alopecia and nausea, vomiting, hypertension, or gamma-glutamyltransferase elevations which were DLT under circumstances described below); any toxicity (except anaemia, nausea, vomiting and alopecia) not resolved to grade 1 or less or baseline within 5 weeks from the first day of study drug administration in course one and anaemia not resolved to grade 2 or less within 5 weeks from the first day of study drug administration. Nausea or vomiting were considered DLT only when occurring despite the use of standard anti-emetics. Hypertension was considered DLT if not manageable with up to 2 oral anti-hypertensive drugs, or associated with evidence of end-organ damage or grade 4 in severity. Gamma-glutamyltransferase (gamma-GT) elevations were only to be considered DLTs if grade 4.

Initially 3 to 4 patients were enroled in a dose group. If DLT was not observed, patients were enroled at the next higher dose level based on the dose escalation scheme described above. If DLT occurred in 1 patient, additional patients were enroled in the dose group to reach a total of 6–7 patients. If no further DLT was observed (1 DLT/6–7 patients), patients were enroled at the next higher dose level. If DLT occurred in 2 or more patients in a dose group, 8 additional patients (for a total of 11–15 patients, depending on the initial number enrolled) were to be enroled in the next lower dose group for the determination of the MTD and oral bioavailability. This lower dose would be the MTD provided <3 of 11–12 patients or <4 of 14–15 patients experienced DLT.

2.5. Pharmacokinetics assessment

Serial blood samples were collected for the ZK 304709 pharmacokinetic determination before and after dosing on days 1 and 7 (pre-dose, 0.5, 2, 4, 6, 10, 12 and 24 h). Additional blood samples were taken on days 14 and 21. Whole blood and plasma (270 and 360 mg dose groups only) samples were analysed using a validated liquid chromatography/mass spectrometry (LC-MS/MS) method. The isolation of the drug substance and the internal standard ($^{13}C_6$-ZK 250765) from whole blood was achieved by solid-phase extraction. After drying the extracts, the samples were reconstituted in 25 μl methanol/water (50:50) and set up for LC-MS/MS analysis. Samples below the lower limit of quantification were set to zero. Non-compartmental pharmacokinetic analyses were done using Kinetica (Thermo Electron Corporation). Pharmacokinetic results are expressed as ZK 250765, the smallest molecular entity of the administered ZK 304709.

Additional blood samples were taken to prepare plasma for protein binding experiments in order to determine the unbound fraction of ZK 304709. For each patient selected plasma samples from treatment day 7 (aiming at C_{max}, $C_{max}/2$ and $C_{max}/4$) were spiked with ^{3}H-ZK 304709. Following an incubation period of 1 h at 37 °C free and protein bound fractions were separated by ultrafiltration.

2.6. Biomarker assessment

Soluble proteins that may be correlates of epithelial cell death or of endothelial cell angiogenic activity and/or pharmacodynamic inhibition of angiogenic growth factor receptor signalling were chosen as exploratory biomarkers. Biomarker determination was conducted on leftover plasma samples for pharmacokinetic and/or safety assessment. Available samples were from 180 mg, 270 mg and 360 mg dose groups and were taken prior to treatment ($n = 12$), on day 1 ($n = 14$), day 7 ($n = 12$) and days 14 and 21 ($n = 5$ and 4, respectively) For non-invasive monitoring of epithelial cell death, total soluble CK18 was determined using the M65-ELISA kit and the caspase-cleaved CK18-Asp396 neo-epitope, which is indicative of epithelial cell apoptosis, was measured using the M30-Apoptosense ELISA kit (both from PEVIVA AB, Bromma, Sweden). Soluble truncated forms of the angiogenic growth factors VEGFR-1, VEGFR-2 and angiopoietin receptor Tie2 are found in serum as a result of their shedding from endothelial cells. Their abundance in plasma samples was determined using Human Soluble VEGF R1/Flt–1–, Human sVEGF R2/KDR- or Human Tie-2-Quantikine ELISA Kits (all from R&D

Systems GmbH, Wiesbaden-Nordenstadt, Germany). Assays were calibrated against recombinant proteins consisting of the full-length extracellular domain of the respective receptors. Results obtained were evaluated with respect to tumour entity, dosing group and treatment duration.

2.7. *Response assessment*

Tumour response was assessed using CT scans after the 2nd and 4th treatment course and during the follow-up visit. The Response Evaluation Criteria in Solid Tumours (RECIST) were used to categorise response.[3] If a patient was treated for more than six courses, a further tumour evaluation was to be performed after every second course.

3. Results

3.1. *Patients*

Fifty four patients were screened, and 40 were enroled and treated at three centres. Of the 40 treated patients, 3 completed the study as planned with at least six treatment courses (Table 1). The remaining 37 patients discontinued the study pre-maturely due to disease progression (19 patients), adverse events (11), death (4) and withdrawal of consent (3). All 40 patients who received at least one dose of the study drug were included in the full analysis set used for analyses of safety, pharmacokinetics and pharmacodynamics. The full analysis set consisted of 40 Caucasian patients (15 female and 25 male). Their median age was 63 years for males and 64 years for females. The patients' diagnoses were colorectal cancer (13 patients), carcinoma of the intrahepatic bile duct system (4), lung cancer (4), pancreatic cancer, oesophageal cancer (3 patients each), ovarian cancer, cancer of the retroperitoneum, renal cell cancer, gallbladder cancer (2 patients each), adenocarcinoma of anastomosis after Billroth II operation, head and neck cancer, cervical cancer, thyroid cancer, mesothelioma, (1 patient each). Six patients had WHO performance status of grade 0, 3 patients had a WHO performance status of grade 2 and the remainder had WHO performance status of grade 1 (31 patients). Prior therapy included chemotherapy or hormonal therapy in 33 patients, surgery in 27 patients and radiotherapy in 7 patients (Table 1). Patients were treated at 8 dose levels ranging from 15 mg to 360 mg, with 3–7 patients per dose level (Table 2).

Table 1 – Baseline patient characteristics

Variable	No. of patients
Male: Female	25:15
Median age	
Male	63
Female	64
Performance status	
WHO 0	6
WHO 1	31
WHO 2	3
Tumour types	
Colorectal	13
Biliary/gallbladder/pancreatic	10
Oesophageal	3
Lung	4
Mesothelioma	1
Ovarian	2
Retroperitoneal	2
Renal	2
Head and neck	1
Cervical	1
Thyroid	1
Previous treatments	
Chemo- or hormonal therapy	33
Surgery	27
Radiotherapy	7

Table 2 – Dose escalation scheme

Dose level	Dose (mg)	No. of patients
1	15	3
2	25	4
3	35	6
4	45	3
5	90	3
6	180	7
7	270	7
8	360	7

3.2. *Pharmacokinetics*

Since the study was terminated before reaching therapeutic exposure the absolute bioavailability of ZK 304709 was not studied. In whole blood, C_{max} and AUC (0-24 h) increased dose proportionally until the 90 mg dose group. Thereafter, no consistent increases with further dose escalation were seen. Maximal blood concentrations occurred around 2 h after administration up to a dose of 90 mg. At higher dosages, there appeared to be slightly delayed absorption with maximal blood concentrations occurring approximately 4 h after administration. The pharmacokinetic exposure parameters showed a high variability between patients (e.g. geometric coefficient of variation for AUC (0–24 h) generally >80%). The comparison of mean concentration-time profiles between day 1 (Table 3) and day 7 (Table 4) suggested that no or only minor accumulation of ZK 250765 took place after multiple daily dosing with ZK 304709 (Fig. 1). Blood concentrations of ZK 250765 exceed plasma concentrations by about 15- to 20-fold. No obvious time dependency in the blood/plasma ratio following single and multiple administration of ZK 304790 was observed The unbound fraction of ZK 250765 in plasma was about 7% and was neither dependent on whole blood nor on plasma concentration.

3.3. *Pharmacodynamic results*

With respect to tumour evaluation, no partial responses (PRs) or complete responses (CRs) were observed. Best overall response according to RECIST could be determined for 28 of 40 patients, with 16 patients having stable disease (SD) and 11 patients having progressive disease (PD); 1 patient was not evaluable.

Table 3 – Pharmacokinetic parameters of ZK 250765 on day 1 of treatment

Dose (mg)	No. of patients	Whole blood		Plasma	
		C_{max} (ng/ml)	AUC (0–24 h) (h ng/ml)	C_{max} (ng/ml)	AUC (0–24 h) (h ng/ml)
15	3	524 (77.1%)	4208 (25.4%)	nd	nd
25	4	754 (52.1%)	6898 (40.0%)	nd	nd
35	6	832 (73.9)	7229 (98.3%)	nd	nd
45	3	1103 (67.5%)	8591 (84.7%)	nd	nd
90	3	2477 (47.4%)	19367 (42.5%)	nd	nd
180	7	2016 (46.4%)	18798 (61.4%)	nd	nd
270	7	2215 (131%)	23883 (99.2%)	166 (168%)[a]	1653 (131%)[a]
360	7	2303 (128%)	27552 (128%)	189 (188%)	1932 (173%)[a]

The geometric mean with the geometric coefficient of variation (in parentheses) are given C_{max} = maximum plasma concentration.
AUC(0–24 h) = Area under the concentration–time curve from 0 h data point up to 24 h post-administration.
a N = 6.

Table 4 – Pharmacokinetic parameters of ZK 250765 on day 7 of treatment

Dose (mg)	No. of patients	Whole blood		Plasma	
		C_{max} (ng/ml)	AUC (0-24 h) (h ng/ml)	C_{max} (ng/ml)	AUC (0–24 h) (h ng/ml)
15	3	554 (76.0%)	4608 (15.2%)	nd	nd
25	3 of 4	790 (42.5%)	8395 (81.6%)	nd	nd
35	5 of 6	1102 (53.4%)	8573 (90.3%)	nd	nd
45	3	1030 (91.1%)	9832 (126%)	nd	nd
90	3	3546 (37.4%)	26664 (26.6%)	nd	nd
180	6 of 7	2347 (90.3%)	17542 (81.2%)[a]	nd	nd
270	7	2461 (115%)	28493 (83.5%)	175 (124%)[b]	1876 (79.9%)[b]
360	3 of 7	2382 (70.5%)	22360 (102%)	146 (72.9%)	1252 (95.4%)

The geometric mean with the geometric coefficient of variation (in parentheses) are given C_{max} = maximum plasma concentration.
AUC(0–24 h) = Area under the concentration–time curve from 0 h data point up to 24 h post-administration.
nd = not determined.
a N = 5.
b N = 6.

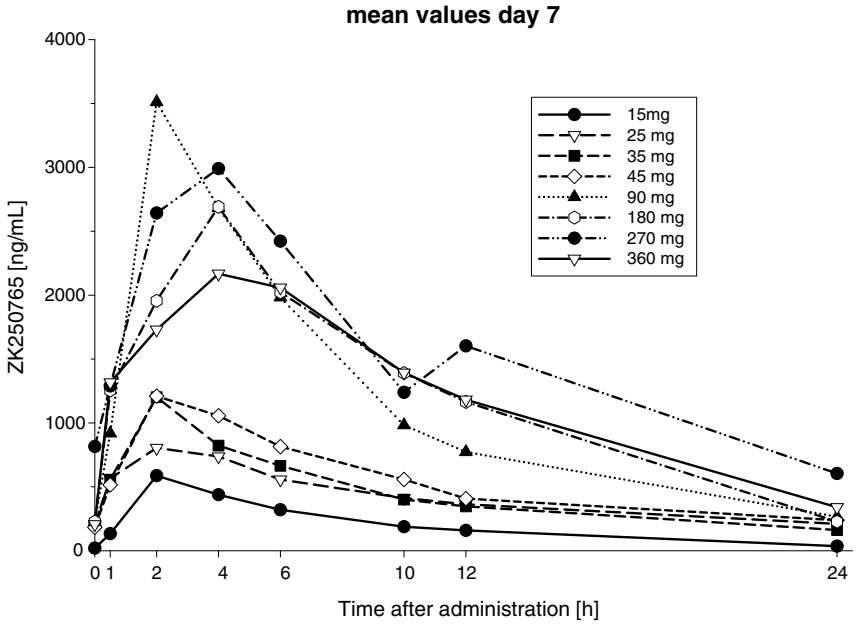

Fig. 1 – Arithmetic mean concentration – time profiles of ZK 250765 (smallest molecular entity of ZK 304709) in whole blood after increasing daily oral doses of ZK 304709, obtained after the last administration (day 7).

No treatment-dependent changes were measured during the first treatment course in exploratory biomarkers capable of differentiating between a cytotoxic and an apoptosis-inducing effect of ZK 307409 (CK 18 and caspase-cleaved CK18)[4,5] and markers that may be correlates of angiogenic activity and/or pharmacodynamic inhibition of angiogenic growth factor receptor signalling (soluble VEGF receptors 1 and 2, soluble Tie2)[6] (non-validated data). This result is not unexpected when taking into account the low exposure to study drug and the pre-mature termination of dose escalation. Further, the small number of patients in this study and the highly heterogeneous spectrum of tumour types precluded a decisive evaluation of CK18 and its fragments as apoptosis biomarkers and of sVEGF R1 and -2 and/or sTie2 as angiogenesis biomarkers for monitoring an effect of ZK 304709. However, when compared to the range reported for control serum, baseline concentrations from pre-treatment samples ($n = 12$) were elevated in 75% of patients with respect to epithelial cell death (Cytokeratin 18) and in 92% with respect to sVEGFR1, supporting their potential to serve as biomarkers in future trials.

3.4. *Safety*

The majority of the safety findings in this study were felt to be consistent with the advanced disease status of the patients, and involvement of the study drug in most cases could not be concluded from this study. The most frequent drug-related AEs were nausea (58%), vomiting (55%), diarrhoea and lymphopaenia (both 35%), fatigue (23%), anaemia (20%), lethargy (18%), anorexia (13%), and dizziness (10%). Sixteen drug-related AEs with a maximum NCI CTC grade of 3 and two drug-related AEs with a maximum grade of 4 were reported in thirteen patients (Table 5). In addition, NCI CTC grade 4 AEs unrelated to study drug were reported in two of these patients (pneumonia and depressed level of consciousness, 35 mg dose level; lower respiratory tract infection, 15 mg dose level), and in one additional patient (biliary sepsis and respiratory failure, 360 mg dose level). DLTs were observed in 5 patients and consisted of intermittent supraventricular tachycardia (35 mg dose level), nausea and vomiting (180 mg dose level), suspected gastrointestinal bleeding (270 mg dose level), diarrhoea (360 mg dose level) and fatigue (360 mg dose level) (Table 6). The study was terminated prior to the MTD being determined.

No clear dose dependency for any toxicity was observed. Renal and hepatic parameters showed no consistent or dose-dependent changes. Overall, abnormal laboratory findings were frequently felt to be in line with the patients' advanced disease status and/or disease progression, and a conclusive attribution to study drug was not possible. In addition, for the majority of patients WHO performance status remained unchanged throughout treatment but deteriorated on subsequent follow-up. Four patients died during this study, 2 due to progression of disease, one due to an unknown cause with suspicion of gastrointestinal bleeding which was considered possibly study drug related, and one due to cholangiogenic sepsis which was considered unlikely to be study drug related.

4. Discussion

In this study, the maximum blood concentration and AUC (0-24 h) for ZK 250765 that were achieved after oral administration of ZK 304709 increased less than dose proportionally, especially at dosages >90 mg. These parameters also showed high inter-individual variability. Such observations are typical when absorption is dose-limited either because of limited drug solubility in gastrointestinal fluids (which is known for ZK 304709), or because of the saturation of intestinal transport pathways (unknown for ZK 304709), which lowers the fraction of the dose that is absorbed. Non-linear pharmacokinetics are also observed for drugs that exhibit concentration-dependent plasma protein binding. Saturable plasma protein binding is not thought to have contributed to the results seen for ZK 304709, since binding was not too strong (unbound concentration about 7%) and independent of concentration.

ZK 304709 is a potent inhibitor of human carbonic anhydrase, especially of type II of this enzyme. However, it seems

Table 5 – Drug-related AEs of at least NCI CTC grade 3

Toxicity	No. of patients	Dose (no. of patients)
NCI CTC Grade 3		
Lymphopenia	6	15 mg (1), 35 mg (2), 270 mg (3)
Nausea	2	35 mg (1), 180 mg (1)
Vomiting	2	35 mg (1), 180 mg (1)
Supraventricular tachycardia	1	35 mg
Diarrhoea	1	360 mg
Fatigue	1	360 mg
Hypophosphatemia[a]	1	270 mg
Decreased Hb	1	360 mg
Increased INR[b]	1	180 mg
NCI CTC Grade 4		
Dizziness	1	270 mg
Increased cholesterol	1	35 mg

a Formally fulfilled DLT criteria but clinically not evaluated as DLT (transient phenomenon).
b Formally fulfilled DLT criteria but clinically not evaluated as DLT (raised INR possibly due to warfarin therapy).

Table 6 – Overview of DLTs

Dose group (mg)	DLT	First documentation
35	Supraventricular tachycardia	Course 1
180	nausea, vomiting	Course 1
270	Suspected gastrointestinal bleeding[a]	Course 3
360	Diarrhoea	Course 1
360	Fatigue	Course 1

a Patient died at home of a suspected GI bleed. Autopsy was not performed. Although in course 3 it was classed as a DLT due to the potential that an antiangiogenic therapy such as this could have been causally linked to.

unlikely that the observed non-linear behaviour resulted from the saturation of these binding sites in erythrocytes and tissue, since the blood/plasma ratio showed no obvious dependency on time and blood concentration. Nevertheless, this evaluation was restricted to the two highest dose groups, and an increased blood clearance at the higher doses cannot be ruled out entirely. The reasons for the observed lack of dose proportionality and the high inter-individual variability remain unclear. However, dose-limited absorption due to the poor solubility/physiochemical properties of the compound seems to be the most likely underlying reason.

5. Conclusion

Taking into account the early termination of the study before the MTD could be determined, we consider the main toxicities of ZK 304709 in humans to be nausea, vomiting, diarrhoea and possibly fatigue, and in general the drug appeared to be well tolerated. In whole blood, Cmax and AUC (0–24 h) increased roughly dose proportionally until the 90 mg dose group. Thereafter, no consistent increases with further increases of dose were seen. Dose-limited absorption due to the poor solubility/physiochemical properties of the compound seems to be the most likely underlying reason. An assessment of the pharmacodynamic effect and potential efficacy of ZK 304709 was not possible due to the low exposure that was achievable. The small number of patients and the heterogeneous tumour spectrum precluded a decisive evaluation of CK18 and its fragments as apoptosis biomarkers and of sVEGF R1 and -2 and/or sTie2 as angiogenesis biomarkers for monitoring ZK 304709. The results of this study suggest that the development of an improved formulation is necessary to overcome the limited and variable exposure in patients.

Conflict of interest statement

Doctors Kristin Kowal and Herbert Wiesinger are employed by Bayer Schering Pharma A G Berlin. Dr. Candice McCoy is employed by Bayer Healthcare Pharmaceuticals, Seattle, US. There are no other conflicts of Interest.

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